Traditional, histopathological and molecular diagnosis of sarcocytosis in slaughtered sheep in Al-Diwaniyah province, Iraq

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Abstract

The presented investigation was performed to identify Sarcocystis species, in an intermediate-host parasite with zoonotic importance, infection in slaughtered sheep identified by traditional, histological, and molecular methods in Al-Diwaniyah Province, Iraq. Here, 70 intercostal and esophageal muscle samples 10-40 gm each were collected from slaughtered sheep. The samples were examined for detection of the Sarcocystic bradyzoites using microscopic, histological, 18S rRNA gene-based polymerase chain reaction (PCR) and PCR-product-dependent gene sequencing methods. The results of the microscopic method revealed that 65 (93%) of the muscle samples contained bradyzoites. The histopathological picture showed the presence of two microsarcocysts with different morphological thin or thick wall sizes. The PCR demonstrated that 68/70 (97%) of the samples were positive for the occurrence of the parasite DNA. According to the NCBI-based websites, the Phylogenetic analysis revealed that the isolates from the current study were closely similar in their nucleotide sequences with isolate 13-16. The present data indicate high infection levels by Sarcocystis spp. in slaughtered sheep, which could bring alarm for public health importance.

Introduction

As one of the Parasites of food-borne illnesses, Sarcocystis are intracellular protozoa. Until recently, 196 genuine species of Sarcocystis have been identified, and each of them is very selective regarding the types of hosts it can live on. Cysts are produced by parasites and may be seen in the striated muscles and the brains of pigs, cattle, and sheep (1-5). Eating raw or undercooked meat carrying sarcocystosis may spread infections of S. hominis, S. heydorni, and S. suihominis. Both S. hominis and S. suihominis have been designated as zoonotic risks by the European Food Safety Authority (6-12). Sheep may be infected with Sarcocystis through drinking contaminated water or eating wild feed containing the parasite's sporocysts, leading to weight loss, abortion, early delivery, and even death (13-16). Most species of Sarcocystis need not one but two different hosts during their life cycles, with carnivores serving as the final hosts and herbivores acting as intermediate hosts. Only four parasitic species have been confirmed to infect sheep: the non-pathogenic parasitic species (macroscopic) S. gigantea and S. medusiformis transferred by felids, and the pathogenic parasitic species (microscopic) S. tenella and S. arieticanis spread by canids (17-21). Sarcocystis spp. Infects various animals, especially mammals, birds, and fish. The final host is responsible for the sexual phases of gametogony and sporogony. In contrast, the intermediate host is responsible for the asexual phases of merogony and cyst development (22,23). Sarcocystis infections in intermediate hosts are often asymptomatic, but severe instances have been observed, resulting in the host's fatigue, loss of appetite, diarrhea, loss of weight, muscular twitching, and in very exceptional instances, mortality. Muscle tissue cysts carrying bradyzoites are a characteristic feature of the degenerative alteration known as sarcocytosis, which occurs in the intermediate host.
Infections by *Sarcocystis ovicanis* or *Sarcocystis neurona* has been linked to rare cases of encephalitis in sheep and horses (24,25).

The presented investigation was performed to identify *Sarcocystis* species, in an intermediate-host parasite with zoonotic importance, infection in slaughtered sheep identified by traditional, histopathological, and molecular methods in Al-Diwaniyah Province, Iraq.

**Materials and methods**

**Samples**

The presented work collected 70 intercostal and esophageal muscle samples (10-40gm/each) from slaughtered sheep from April-December, 2022. The samples were cooled-box-transported to the Laboratory of Parasitology, College of Veterinary Medicine, University of Al-Qadisiya, Al-Diwaniyah City, Iraq.

**Microscopic examination**

The samples were examined for Sarcocystic bradyzoites and cysts using squeezing, trichoscopy, and solution analysis methods described by Al-Bayatee (26) and Al-Dulaimi (27).

**18S rRNA gene-based PCR**

18S rRNA gene-based PCR was performed using primers, Sar-F1: GCACCTTGATGAAATTCTGGCA and Sar-R1: CACCACCCATAGAATCAAG, followed by Stecher et al. (13). The QIAamp DNA Mini Kit (Qiagen, Germany), with steps from the kit itself was employed for the bradyzoite DNA extraction. A 1.5% agarose gel was placed under an electrophoresis process for 60mins at 100 volts and 80Amp. The gel, after that, was visualized under a UV-based imager. Stecher et al. (28) employed the methods in the current section.

**18S rRNA-PCR product-based sequencing**

The PCR-product-dependent gene sequencing method sends the PCR-purified products to Macrogen (Korea) for sequencing. The sequences were processed using MEGA X software and NCBI websites to complete the analyses and build a phylogenetic tree.

**Results**

**Microscopic test**

The results of the microscopic method revealed that 65 (93%) of the muscle samples contained bradyzoites (Figure 1).

**Histopathological findings**

The pathohistological picture showed the presence of two microsarcocysts with different morphological thin or thick wall sizes (Figure 2).

**18S rRNA gene-based PCR findings**

The PCR demonstrated that 68/70 (97%) of the samples were positive for the occurrence of the parasite DNA (Figure 3).
Table 1: Homology sequence identity (%) in local Sarcocystis spp in sheep

<table>
<thead>
<tr>
<th>Obtained Accession number</th>
<th>Identical to</th>
<th>GenBank Accession number</th>
<th>Country</th>
<th>Identity (%)</th>
<th>Host</th>
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<td>OP835896</td>
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<td>MT560372</td>
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<td>sheep</td>
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<tr>
<td>OP835898</td>
<td>Sarcocystis tenella</td>
<td>MK420019</td>
<td>Norway</td>
<td>99.80</td>
<td>sheep</td>
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<tr>
<td>OP835899</td>
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<td>MG515221</td>
<td>Egypt</td>
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<td>sheep</td>
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</tr>
</tbody>
</table>

Figure 3: Image of agarose gel electrophoresis. It shows the 18S rRNA gene-based piece amplification of Sarcocystis spp. of sheep. 1-16: Positive samples. C: Negative control (No DNA). M: Molecular marker.

Figure 4: Phylogenetic tree of Sarcocystis spp in sheep based on the 18S rRNA gene.

Discussion

Similar to the earlier findings from Baghdad 91.93% (26), this research found a prevalence of Sarcocystis infection in sheep of 93%. And in Mongolia, the sheep have an infection rate of 97%, a 100% in Iran's sheep population, and 84 % in American sheep (29). Molecular methods, such as PCR amplification of species-specific 18s rRNA, are necessary for identifying species in this genus, which cannot be done only by microscopy. These results, at 97%, show that molecular diagnosis for sarcocystosis has a greater infection rate than traditional methods. Likewise, 90.78 % of the sheep samples in Karbala Province (30), Iraq, were infected with the parasite (31-33).

The Sarcocystis includes Sarcosporidia, known for their versatility in where they invade and grow inside the intermediate host. In addition, investigational transfer of the muscle cysts from sheep to dog puppies revealed the coccidian character of Sarcocystis, where the parasite was found at a higher incidence (34). Sarcocysts, only seen in the muscular parts of intermediate hosts, were characterized as evidence of a wide variety of host species and global dissemination. The current research demonstrated that natural infection rates in the tested hosts' skeletal muscles and organs varied. These findings are consistent with those found previously in Riyadh (35-37).

Alternatively, other nations reported lower levels. This research demonstrated a very varied rate of Sarcocystis distribution throughout several sheep organs. Oocyst exposure, changes in host immunity owing to abnormalities in the environmental and nutritional state, and parasite speciation are all possible causes for the observed variances in intensity among the tested hosts, as revealed by different studies (38). Other reports of detecting Sarcocystis spp. in different countries, such as Saudi Arabia, Tunisia, and China (39-41).

Conclusion

The present data indicate high infection levels by Sarcocystis spp. in slaughtered sheep, which could bring alarm for public health importance.
Acknowledgments

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Conflict of interests

The authors have not received any funding or benefits from industry, financing agency, or elsewhere to conduct this study.

References


الطرق التقليدية، النسيجية المرضية والجزئية

تشخيص الحويصلات الصنوبرية في الأغنام المذبوحة

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هديل هادي جواد و غيداء عباس جاسم

الخلاصة

تم إجراء البحث المقدم هنا لتعرف على وجود خمج الحويصلات الصنوبرية، وهو طفيلي المضيف الوسيط ذو أهمية للأمراض حيوانية المصدر، في الأغنام المذبوحة التي تم تحديد الخمج بالطرق التقليدية والنسيجية والجزئية في محافظة الديوانية، العراق. تم جمع 65 عينة من العضلات احتوت على الـحوينات بطيئة التكاثر، اضطررت الصورة المرضية النسجية وجودة أكياس صغيرة ذا حجم جدار رقيق في كل منهما من الأغنام. تم فحص العينات لوجود الحويصلات بطبية التكاثر باستخدام تفاعل البلمرة المتسلسل للجين الرايبوسومي وطرق دراسة القواعد النيتروجينية وطرق دراسة تعاقب القواعد النيتروجينية لمنتجات تفاعل البلمرة المتسلسل. أظهرت نتائج الطرق المجهرية أن 65 (97.5)٪ من عينات العضلات احتوت على الحويصلات بطبية التكاثر. أظهرت الصورة المرضية النسجية وجودة أكياس صغيرة ذات حجم جدار رقيق أو سميك. أظهر تفاعل البلمرة المتسلسل أن 65 (97.5)٪ من العينات كانت إيجابية لوجود الحمض النووي للطفيلي في العينات المفحوصة. كنتيجة تطور النسب أن العزلات من الدراسة الحالية كانت متشابهة إلى حد كبير في متوسطات القواعد النيتروجينية مع عزلات من النرويج ومصر وإيران. تشير البيانات الحالية إلى مخاطر إصابة هذاطفيلي الحويصلات الصنوبرية. الأغنام المذبوحة، الأمر الذي يمكن أن ينذر بأهمية الصحة العامة.